

FIG. 1

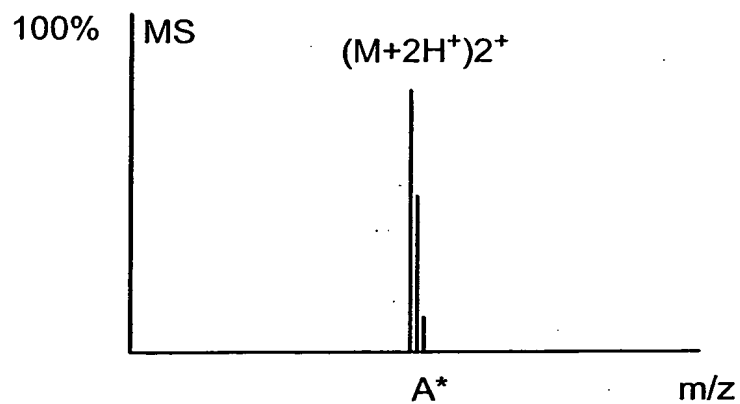


FIG. 2

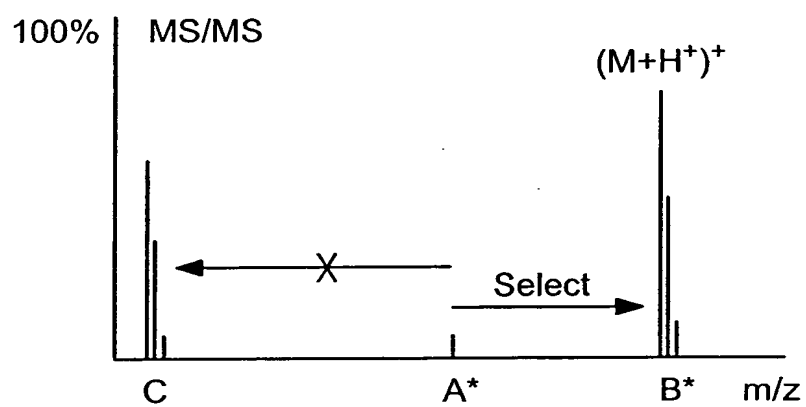


FIG. 3A

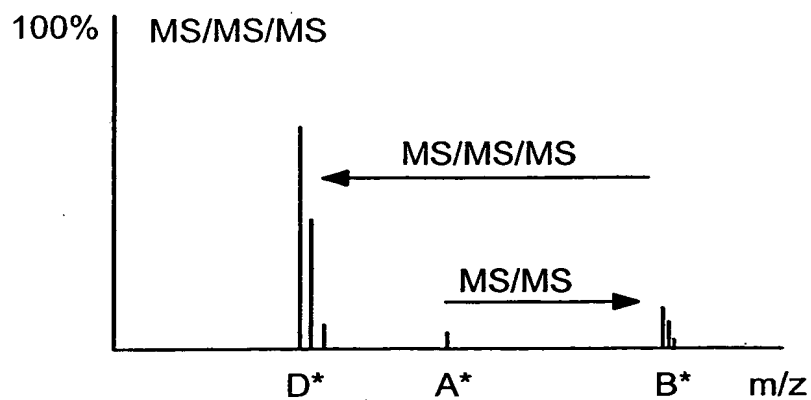


FIG. 3B

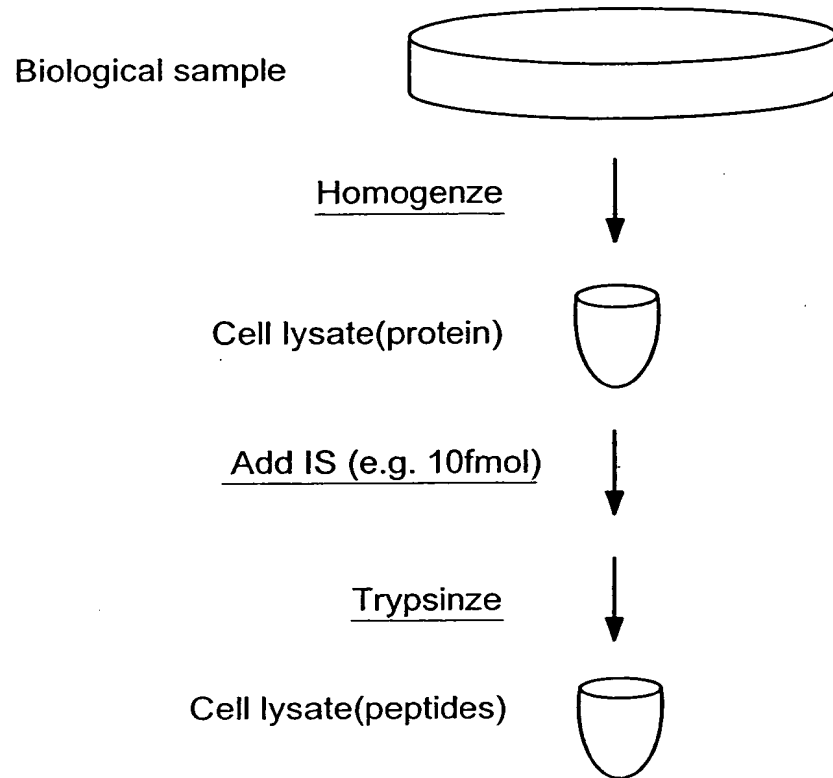


FIG. 4A

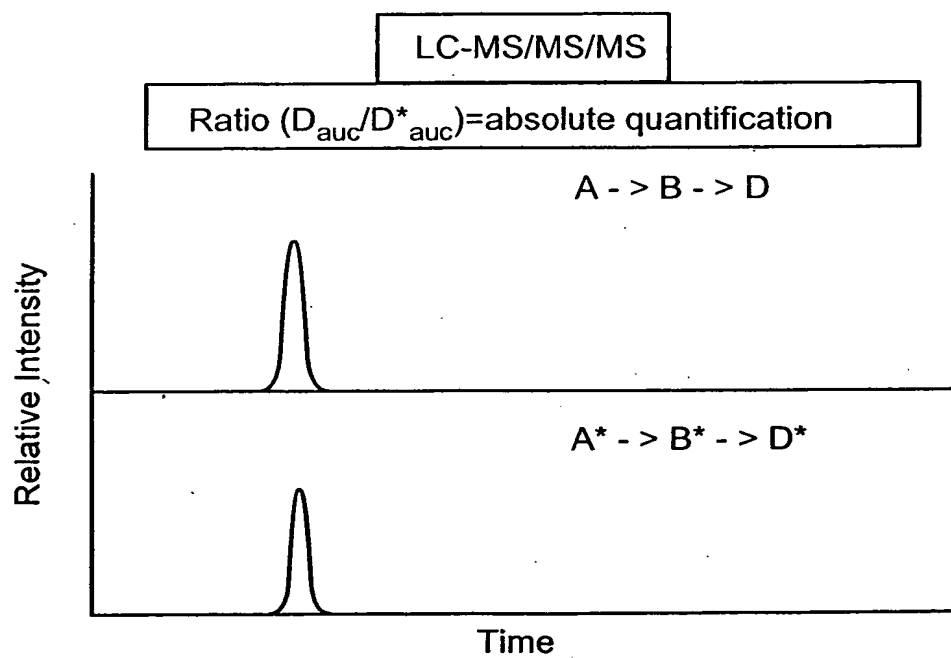
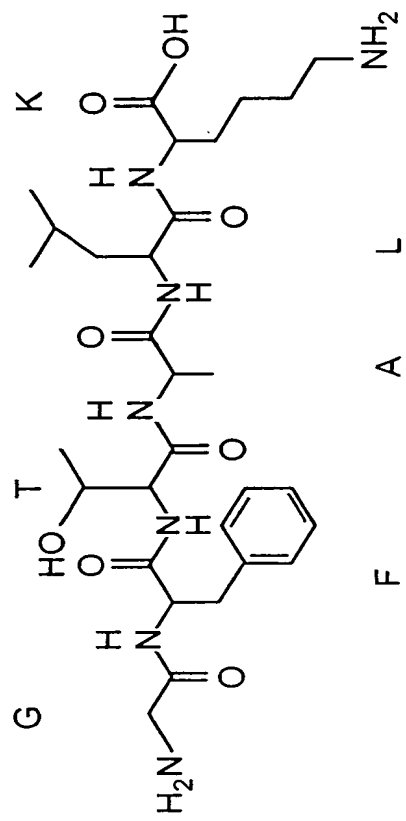


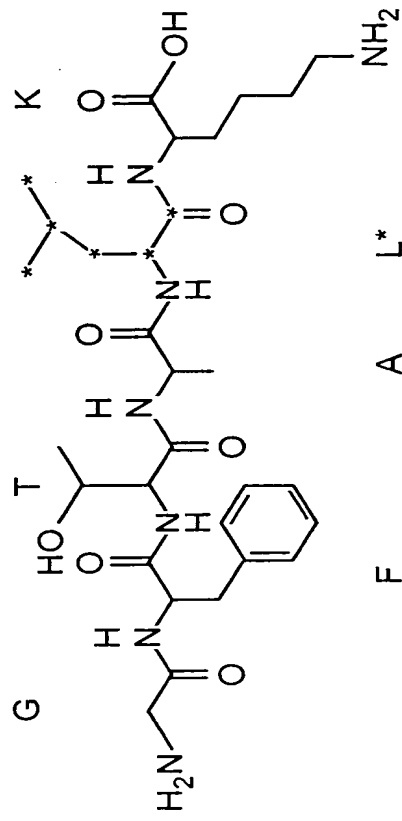
FIG. 4B



Native tryptic peptide

GFTALK

MW: 635.364



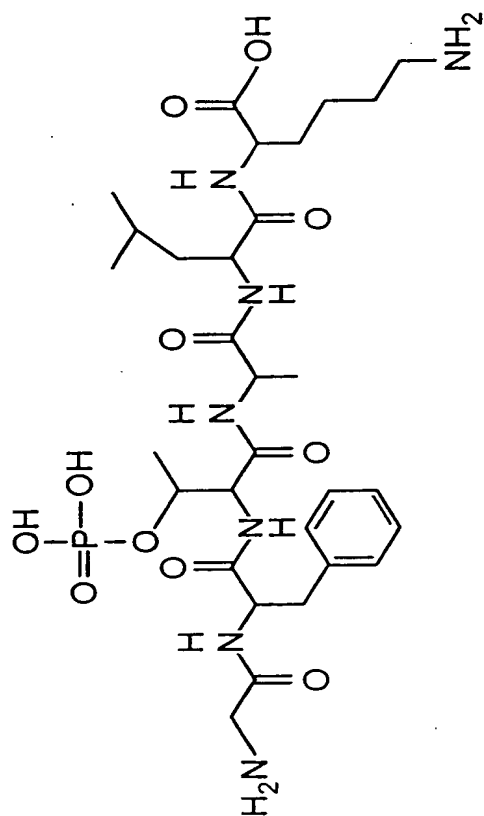
AQUA Internal Standard

GFTAL*K

MW: 641.364

* = stable isotope (e.g. ^{13}C)

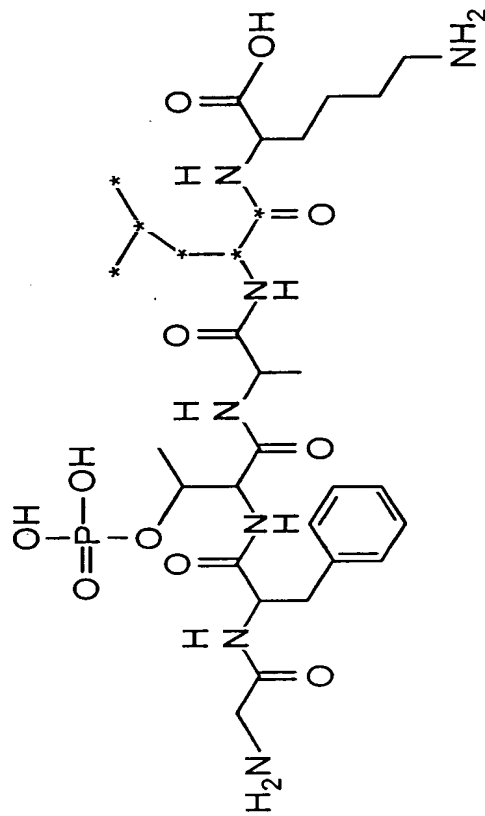
FIG. 5A



Native phosphopeptide

GF(pT)ALK

MW: 715.754

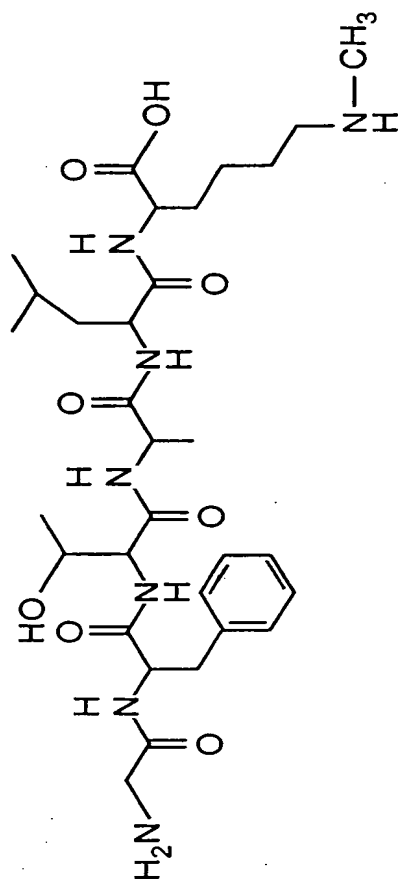
AQUA phosphopeptide
Internal Standard

GF(pT)AL*K

MW: 721.75

* = stable isotope (e.g. ^{13}C)

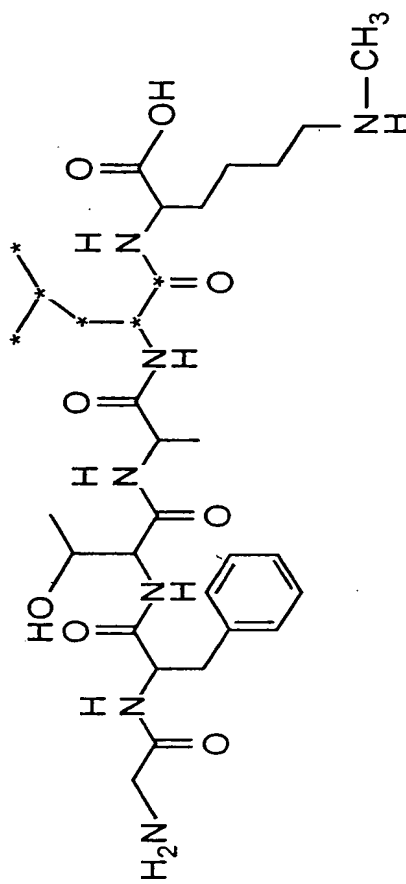
FIG. 5B



Native methylated peptide

GFTAL(mK)

MW: 649.79



AQUA methylated peptide
Internal Standard

GFTAL*(mK)

MW: 655.79

* = stable isotope (e.g. ^{13}C)

FIG. 5C

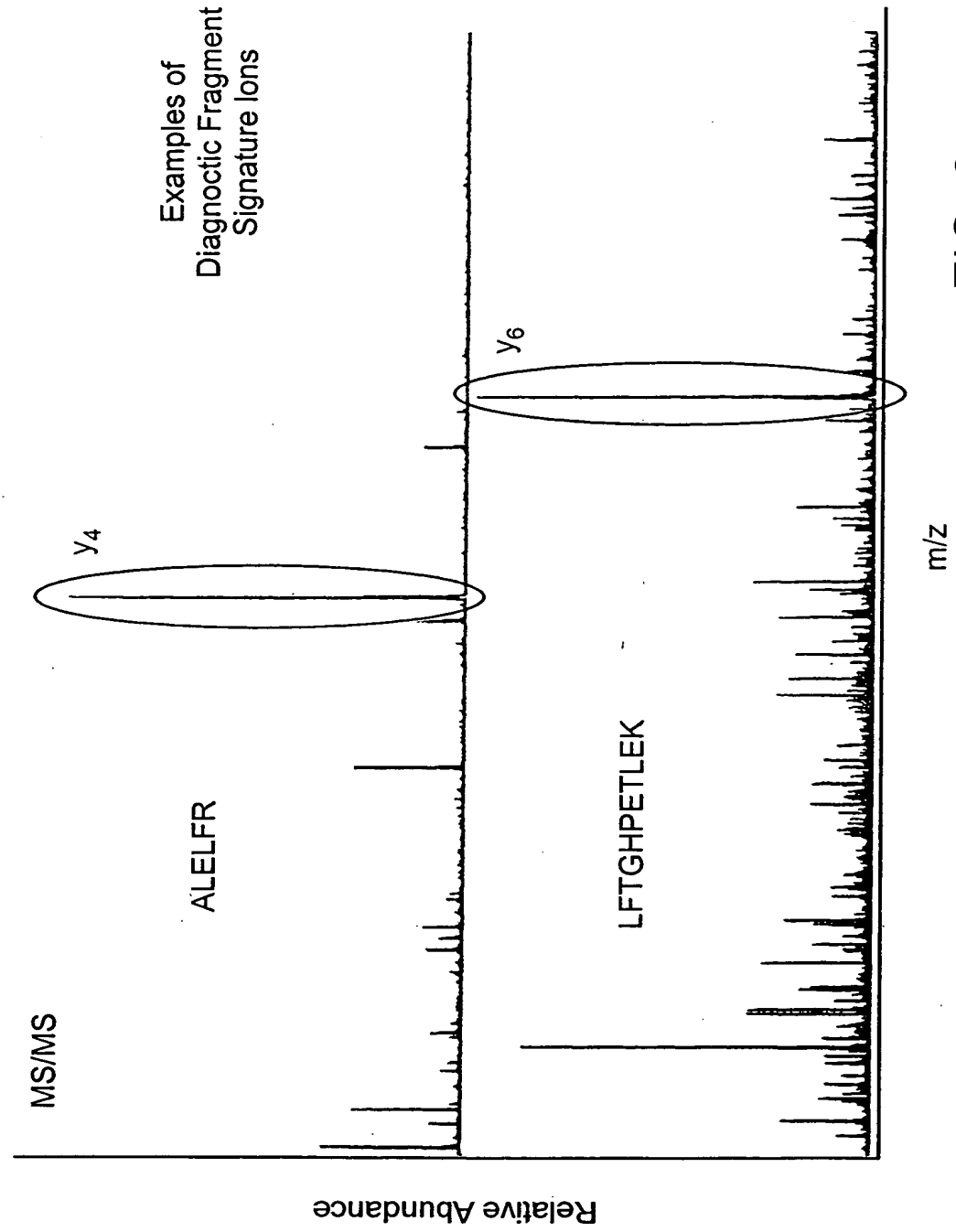


FIG. 6

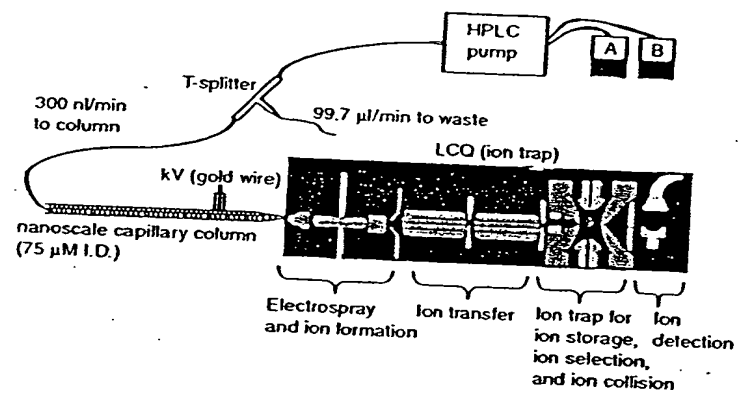
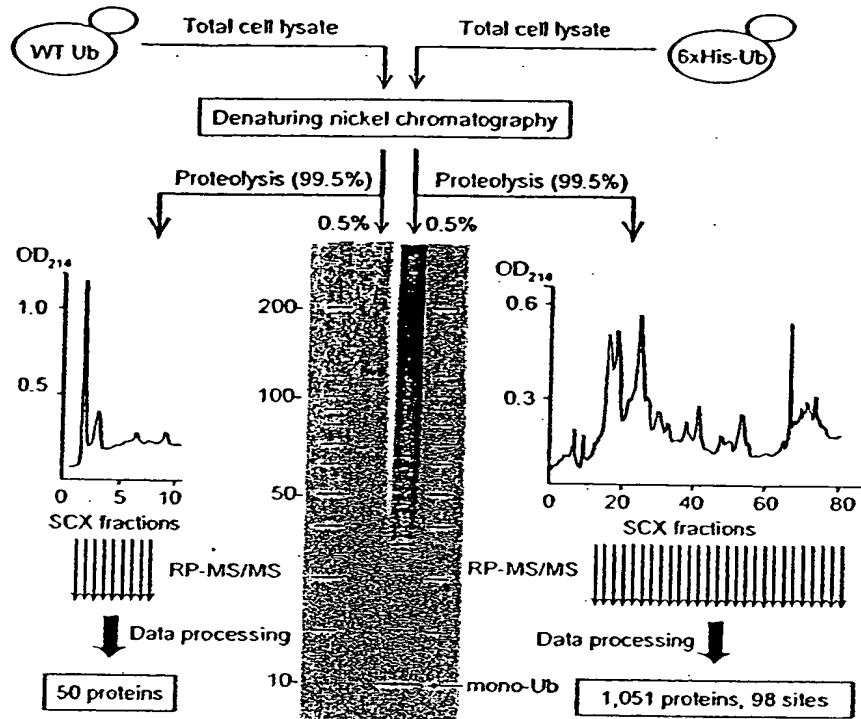


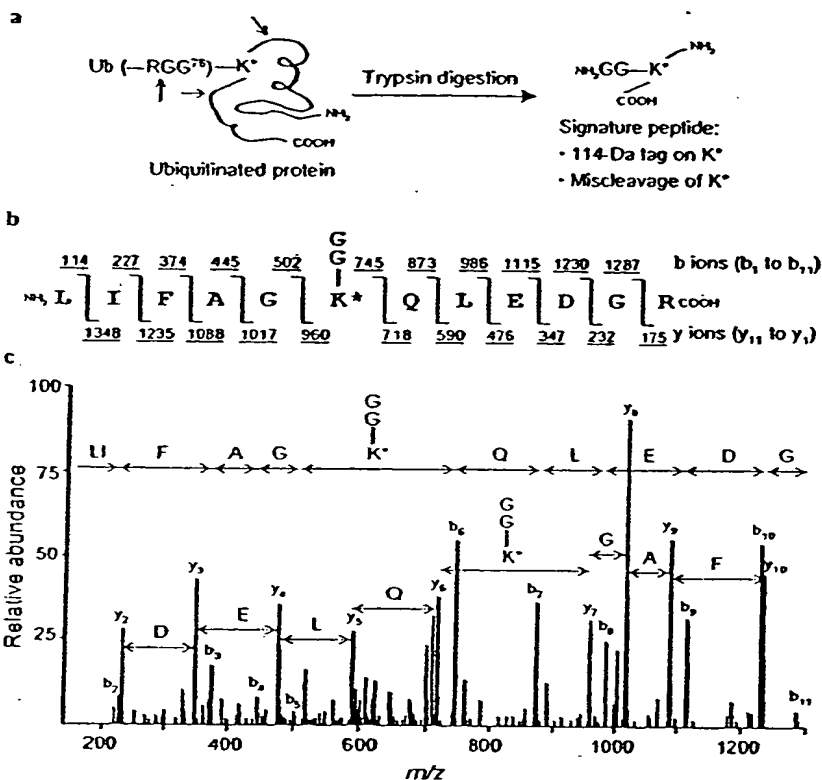
Figure 7

Figure 8



10/506877

Figures 9A-9C



Figures 10A-10C

a

Name	#sites	
Ubiquitin	5	MPFI TSPVA KNSHSELSET ILNOSKQPF QSPFDQLGS AQRRRSSTI RHALSSTLLGS
ECM21	5	AMHSPAVLN NITKCGNNG N RSSNTDAQ LLGQKQKOP PPNARRHSTT AI QCSI SDGA
SAM2	4	TTTPRSSTSD TNRRTSGRLS YDQEPRI SGG RYSQ EEDST YLDFDDHNS SAVVSDLS
YHR097C	3	TSLTRANSK KTFEQLI EY LTARGLLPK TVLSNEYLI SI STSGESVF LPTI SSNDCE
HXT7	3	YLSRLNLND GTDDAEADF MGG DQEGN TPLATTAA TESQSI NEN RUTLLRENS
GNP1	3	GDHPGSEEL NTRSVI DSS MSYSI AM V SVQGTFTD MQLCLSRVK VFWTGVPTT
YIL041W	2	KTFNEEFYNA ASKQWLNDE NDLFVPLSI SPDDQM ENN SNDRQRLFK N PTEERLYL
YHL010C	2	DKTKKASLL NAI DYNKTHL YQDQVFLV PVVFSNH PE TI YLPSARVS YRLRLATKA
LSB1	2	NRKGFYRQS NSPQI VSPD SSSLSSTTS SLQLTETESA QAHRI SNTL FSKVQNHUM
ZE01	2	SSHQLNEES GEELFAEYE IXM KTFEPY AVSTANPLY IIRVWISLS YEI SFAQCYV
PHO84	2	SLNKEVPI KI KLAPI QQNC VKR HNSI TE RVTFYSKQIE YEDQITLVA KQFNYEYLD
URA3	2	FASKRRKERS VSLFEI RTKE KGTALREED VENSFNQLE SYSPFDCEED SNGPKERLG
GDH1	2	I TEPII I ETK LKPKYEDLD KRTAQ I PPY Q DAYTSI PN PEHAVANGPS HRRPSM GFE
ERG5	2	SGKCKSHK ENKPYDPK FIKZII ISN QLVKTHRL NTPKRLYLD SLHFSNVYR
ERG3	2	HQLEIMRIS KPDPECSKL RHYEVL DTP I FLVSEQGS QNELPTYDM ATMECKNQV
YGR268C	2	PLSMGDTFG NTPPPPTFE EAI SVFASPI VSPMGPMI M ASYIDLLSI QQLNLSKTS
YMR295C	2	VSQPSGYSID AGVPMNRNS I SNANMGS I SNAFVSGN SOQGVARARA TSVNDRSRFN
		NLDKLLSTPS PYNRSHNSP TRLSQNGT VRI PNATTEN SKIKQNEFTK KGYTLANVXD
		DEEQEG VSS SSADGILLSHG NEPPRYDEI V PLNDEE

b

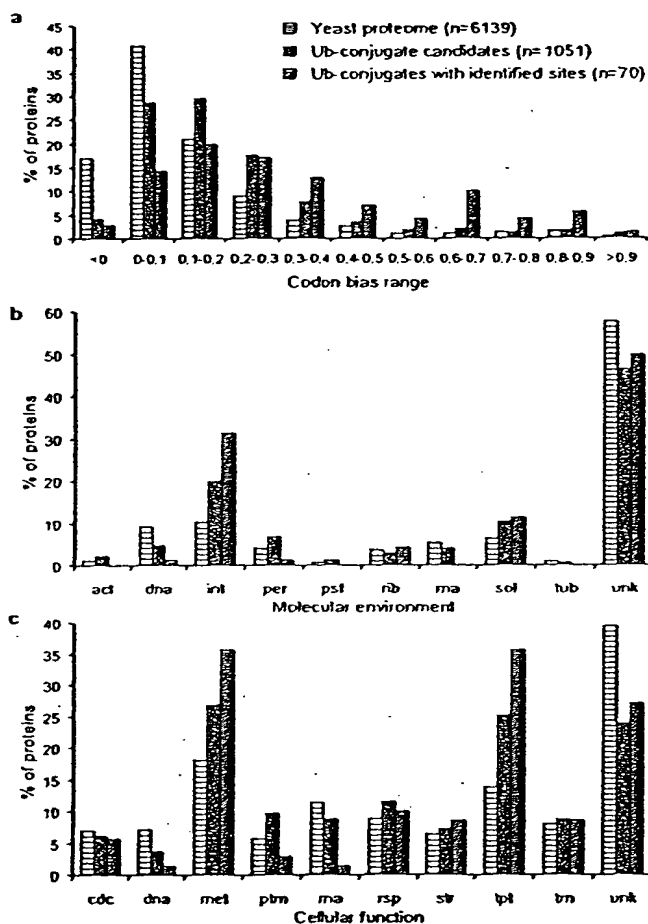
Site in Ub (K)	Signature peptide	SCX fraction	Abundance
48	LIFAGK*QLEDGR	48-56 (9)	high
63	TLSDYNQK*ESTLHLVLR	65-72 (8)	high
11	TLTGK*TITLEVESSDTIDNVK	38-42 (5)	medium
27	TITLEVESSDTIDNVK*SK	41 (1)	low
6	LISEEDLGMQIFVK*TLTGK	38 (1)	low

c

Name	Phosphopeptide sequence	Description of function
ACC1	AVS*YSDLSYVANSQSSPLR	Acetyl-CoA carboxylase
CCC1	GGGGTSELGGSEST*PLLK	Protein potentially in calcium regulation
CH01	DENDGYAS*DEVGGLSR	Phosphatidylserine synthase
CHS1	DDEYDDLNT*IDK	Chitin synthase I
CHS3	NPSTLLPTS*SMFWNK	Chitin synthase III
ECM21	NEES*GEEDFAEYPIK	Protein possibly in cell wall biosynthesis
	HALSS*LLGGANVHSPAVLNNTTK	
	RPS*VIGFLSGHK	
	S*HNS*PTINGLSQANGTVR	
GCD6	EEIDS*EFEDDFEK	Translation initiation factor eIF2B
HSP30	ASGETAHEPEPEAEQAVEDT*A	Heat shock protein located in cell membrane
LYP1	LQVVSHE*DNEDDEEAHYEDK	High affinity lysine-specific permease
MET4	KYS*DNEDDEYDDADLHGFEK	Transcriptional activator
MYO3	RGS*YYHVPLNPVQATAVR	Myosin type I
PHO84	IHDTS*DEDMAINGLER	Inorganic phosphate/H ⁺ symporter
	NNDIESS*PSQLQHEA	
RAD16	SVNYNELS*DDDTAVK	Nucleotide excision repair protein
Ubiquitin	TLSDYNQK	Protein for posttranslational modification
YDR119W	IEEINENS*PLLSAPSK	Member of major facilitator superfamily (MFS)
YDR348C	TNS*FDMPOLNTR	Protein of unknown function
YHR097C	ETVDDDS*ETLNQLQDR	Protein of unknown function
	LPSYEEAAGT*PK	
YOR042W	KNPDEDEFINS*DDDM	Protein of unknown function
	SSGIDEDEVYT*PAEDAKEEEEHPPLPAR	
	EOHHEDS*EEEDSWOFVEK	
YPL019C	HYADLEDHES*S*DEEGTALPK	Vacuolar polyphosphate accumulation

a, Proteins identified containing multiple ubiquitination sites. **b**, Five lysine residues in ubiquitin were found to be sites for (poly)ubiquitination. The number in parenthesis indicates the total fractions in which the signature peptide was detected. **c**, List of phosphorylated peptides from the Ub-conjugates candidates. Phosphorylated residues are indicated by asterisk. Three peptides contained multiple (two) phosphorylation sites. There were 19 proteins identified from 26 phosphopeptides with 29 total phosphorylation sites. Of special interest is the phosphorylation of S⁵⁷ in Ub itself. The sequence of ECM21p is also shown with 5 ubiquitination and 5 phosphorylation sites highlighted. Sixty five other unmodified peptides were also identified from this protein (not shown for clarity).

FIGURES 11A-11C



Comparison of protein expression, environment, and function among the yeast proteome, 1,051 candidate Ub-conjugates, and 70 proteins with detected Ub sites. **a**, Codon bias value (an indicator of protein expression levels with a value < 0.1 indicating likely low-copy-number proteins). **b**, protein molecular environment. act: actin-associated; dna: DNA-associated; int: integral membrane; per: peripheral membrane; psf: protein synthesis factor; rib: ribosome-associated; sol: soluble; tub: tubulin-associated; ma: RNA-associated; unk: unknown. **c**, cellular function. cdc: cell division control; dna: DNA recombination, replication and repair; met: metabolism; ptm: protein posttranslational modification and degradation; ma: RNA transcription, processing and turnover; rsp: responses to environment; str: cellular structure; tpt: transport of proteins and small molecules; trn: protein translation and assembly; unk: others and unknown.

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☒ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER: _____**

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.